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Xu, C; Han, X; Ru, S; Cardenas, LM; Rees, RM; Wu, D; Wu, W; Meng, F

Published in:
Geoderma

DOI:
[10.1016/j.geoderma.2019.01.014](https://doi.org/10.1016/j.geoderma.2019.01.014)

Print publication: 01/05/2019

Document Version
Peer reviewed version

[Link to publication](#)

Citation for pulished version (APA):

Xu, C., Han, X., Ru, S., Cardenas, LM., Rees, RM., Wu, D., Wu, W., & Meng, F. (2019). Crop straw incorporation interacts with N fertilizer on N₂O emissions in an intensively cropped farmland. *Geoderma*, 341, 129-137. <https://doi.org/10.1016/j.geoderma.2019.01.014>

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Crop straw incorporation interacts with N fertilizer on N₂O emissions in an intensively cropped farmland

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Abstract

Nitrogen (N) fertilization and straw incorporation strongly influence nitrous oxide (N₂O) emissions from agricultural fields. An in-situ micro-plot experiment on intensively farmed winter wheat (*Triticum aestivum* L.) was conducted to investigate the source and rate of N₂O emissions from soils following labeled ¹⁵N fertilization with and without straw incorporation. Four treatments, i.e., no N fertilizer and no straw incorporation (N0S0), straw incorporation only (N0S1), N fertilizer only (N1S0), and N fertilization plus straw incorporation (N1S1), were established in the experiment. The N₂O emissions mainly occurred after N fertilization and lasted for approximately 1–2 weeks, accounting for 60%–67% of the wheat seasonal N₂O emissions. Within the 6 days after basal fertilization and 2–4 days after top-dressing, most of the N₂O fluxes (>50%) were derived from fertilizer. Thereafter, soil-derived N₂O dominated the total N₂O emissions and within 10–20 days after N fertilization, fertilizer-derived N₂O became negligible. Fertilizer N and soil N both accounted for 40%–60% of the seasonal N₂O emissions, which may be explained by the high soil N stock due to long-term high N fertilization in the region. This implies the similar roles of soil N pool and fertilizer N in N₂O generation under intensively farmed soils. The N fertilization had a significant priming effect on the turnover of soil N, which contributed 21.0%–28.6% of the seasonal N₂O emissions. During the basal fertilization/first irrigation event, straw incorporation significantly ($P < 0.05$) stimulated CO₂ fluxes both in N-fertilized and non-N-fertilized plots; however, after the top-dressing/second irrigation event, the significant increase of CO₂ fluxes

induced by straw incorporation was only observed in the N-fertilized treatment. Straw incorporation interacted with N fertilization, and tended to enhance N₂O emissions in the basal fertilization and lower N₂O emissions in the top-dressing period. In N-fertilized plots, the seasonal N₂O emissions from straw-incorporated and straw-removed treatments were similar, indicating that straw incorporation enhanced the N supply without increasing the N₂O emissions. Our study highlights that there are significant benefits of straw incorporation to soil fertility improvement; however, the long-term impacts of straw incorporation on greenhouse gas emissions should be further examined.

Keywords: Nitrous oxide; ¹⁵N tracing; Straw incorporation; Nitrogen fertilization; Intensive farming.

1. Introduction

Nitrous oxide (N₂O) is a major greenhouse gas (Ding *et al.*, 2015; Loick *et al.*, 2017), which has 265 times greater global warming potential than CO₂ over a 100-year time horizon (IPCC, 2014). Agricultural soils are the dominant emitters of N₂O, contributing 60% (Smith *et al.*, 2007) and 74% (NCCCC, 2012) of global and Chinese N₂O emissions, respectively. A better understanding of the pattern and sources of N₂O emissions from agricultural soils is therefore essential to develop novel and practical strategies to limit climate change (Kim and Giltrap, 2017).

Northern China is a major intensive agricultural region (Tan *et al.*, 2017; Xu *et al.*, 2017), covering about 3 million ha (Ding *et al.*, 2007) and accounting for 67% and 28% of national wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) production (Zhang *et al.*, 2017b), respectively. High productivity in northern China largely relies on excessive utilization of synthetic nitrogen (N) fertilizer at rates of >600 kg N ha⁻¹ yr⁻¹ (Ju *et al.*, 2009); this high level of N input is likely to result in high N₂O emissions (Zhang *et al.*, 2014b; Omonode *et al.*, 2017) as the N supplied exceeds crop demand (Linguist *et al.*, 2012; Kim *et al.*, 2013; Charles *et al.*, 2017; Song *et al.*, 2018). Reduction in N₂O emissions in northern China could therefore strongly contribute to the mitigation of anthropogenic N₂O emissions at national and global scales (Tan *et al.*, 2017; Xu *et al.*, 2017). Both fertilizer N and soil N pools are responsible for N₂O emissions (Shepherd *et al.*, 2015), so understanding the partitioning of these sources is important both to characterize total emissions and also to allow the precise calculation of emission factors of fertilizer N (IPCC, 2006).

Earlier field studies conducted in northern China presumed that the N₂O emitted from fertilizer N applied in the crop season was the predominant source of total N₂O emissions (Liu *et al.*, 2012; Shi *et al.*, 2013; Ying *et al.*, 2017). However, many in-situ studies using ¹⁵N tracers carried out in Europe (Linzmeier *et al.*, 2001; Garcia-Ruiz *et al.*, 2012), Oceania (Di and Cameron, 2008), and Africa (Gentile *et al.*, 2008) found that soil N could account for as much as 60%–99% of the total N₂O emissions, suggesting that the soil N pool contributed a large proportion of the N₂O emissions. Isotopic analysis using ¹⁵N allows the source and amount of N₂O emissions from fertilizer N to be determined directly (Baggs, 2008; Loick *et al.*, 2017), but most in-situ ¹⁵N tracing studies in northern China have not measured the ¹⁵N₂O flux (Cai *et al.*, 1998; Xu *et al.*, 2000; Cai *et al.*, 2002; Ju *et al.*, 2009) and thus accurately distinguishing the N₂O derived from fertilizer N and soil N pools has not been achieved. Wan *et al.* (2009) used an incubation method to determine the ¹⁵N₂O derived from different N sources; however, their laboratory experiment was incapable of analyzing the effects of natural field conditions (e.g. temperature, precipitation, and plant growth), which were also essential in affecting ¹⁵N₂O emissions (Klumpp *et al.*, 2011). Thus, it is difficult to draw conclusions from the existing literature that quantify the contribution of fertilizer N vs soil N to N₂O emissions in northern China, where excessive fertilization has been implemented for more than three decades (Gu *et al.*, 2017; Huang *et al.*, 2017; Zhang *et al.*, 2017b). In addition, the high biomass production in northern China has generated vast quantities of crop straw and residues (Zhou *et al.*, 2017). A combination of synthetic N fertilizer

with straw incorporation is strongly recommended as an environmentally friendly strategy by researchers (Liao *et al.*, 2015; Zhao *et al.*, 2015; Han *et al.*, 2018) and government agencies (Ministry of Environmental Protection-PRC, 1999; Ministry of Agriculture-PRC, 2015) to improve soil fertility and minimize negative environmental impacts. Incorporation of crop straw is generally believed to have positive effects on soil carbon (C) and N dynamics (Chen *et al.*, 2014; Ghimire *et al.*, 2015; Meng *et al.*, 2017) and on the mitigation of N₂O emissions (Frimpong and Baggs, 2010; Badagliacca *et al.*, 2016). However, results of previous studies on the efficacies of straw incorporation on N₂O emissions were inconsistent, showing either positive (Zhang *et al.*, 2015; Huang *et al.*, 2017), negative (Xia *et al.*, 2014; Yao *et al.*, 2017), or neutral effects (Zhang *et al.*, 2017a). In addition, very few studies have considered the effects of crop straw addition on the source of the generated N₂O (Frimpong *et al.*, 2011; Garcia-Ruiz *et al.*, 2012; Rezaei Rashti *et al.*, 2017; Wu *et al.*, 2017), which would help to quantify total N₂O emissions and inform mitigation actions.

In this study, we used ¹⁵N tracing to evaluate the contribution of the soil N and the fertilizer N to the total N₂O emissions in the intensive farming region of northern China. Measurements under straw-incorporated and straw-removed treatments were also performed to investigate the impact of straw incorporation on N₂O emissions and their sources.

2. Materials and methods

2.1 Study site

The experiment was conducted in Huantai county, Shandong province (36°58'N, 117°59'E), a typical intensively farmed region in northern China (Bai *et al.*, 2011). The region has a temperate monsoon climate (Shi *et al.*, 2013). Annual mean precipitation and air temperature in the region is 543 mm and 12.5°C, respectively (Tan *et al.*, 2017). Prior to the experiment, two crops of winter wheat and summer maize per year had been farmed for about 30 years (since the 1980s). The experiment was conducted in the winter wheat season (Oct. 2015 to Jun. 2016; Fig. 1); the cumulative precipitation, mean air temperature, and mean soil temperature (0–10 cm) during the experimental period were 210 mm, 9.8°C, and 8.4°C, respectively. The soil in the experimental site is classified as aquic inceptisol (calcareous, clay loam; Shi *et al.*, 2013). Soil pH, bulk density, total N content, and soil organic matter content of top layer (0–20 cm) were 7.70 (water/soil = 2.5/1), 1.52 g cm⁻³, 1.00 g kg⁻¹, and 17.4 g kg⁻¹, respectively.

2.2 Experiment design and setup

Four treatments were established in our study: no N fertilizer and no straw incorporated (N0S0), straw incorporation only (N0S1), N fertilizer only (N1S0), and N fertilization plus straw incorporation (N1S1). Each treatment was replicated three times. The resultant 12 microplots (1 × 1 m²) were randomly established in the

experimental area with a path (1 m) between microplots. Each microplot was enclosed with a PVC board, which was inserted into the soil at 1 m depth and the upper edge was 15 cm above the soil surface. Before winter wheat sowing, phosphorus (P) fertilizer (calcium superphosphate; 140 kg P₂O₅ ha⁻¹) and potassium (K) fertilizer (potassium sulfate; 60 kg K₂O ha⁻¹) were broadcasted in all microplots. The topsoil (0–20 cm) was then plowed with a shovel to mix the P and K fertilizer with soil. In the straw-incorporated treatments (N0S1 and N1S1), straw from the previous maize season (0.96 kg m⁻²; C/N: 76:1) was chopped at 3–5 cm and incorporated thoroughly with the soil via plowing. Seeding rate for each micro-plot in the study was consistent with that in local conventional farmland (i.e., 150 kg ha⁻¹; about 330 seeds/micro-plot). All microplots were surrounded by guard rows. After sowing, all microplots were irrigated with 75 mm water. In N-fertilized treatments (N1S0 and N1S1), ¹⁵N labeled urea (125 kg N ha⁻¹; 10.21% atom % ¹⁵N, Shanghai Chem-Industry Institute) was dissolved in the irrigation water and applied uniformly to the microplot as a basal fertilizer. At the jointing stage, irrigation was also applied with 75 mm of water, and an additional 125 kg N (¹⁵N labeled urea) ha⁻¹ was applied as top-dressing. The detailed dates of field management events are shown in Fig. 1.

2.3 N₂O and CO₂ flux measurements

The closed chamber method was used to simultaneously measure the N₂O and CO₂ fluxes (Shi *et al.*, 2014; Tan *et al.*, 2017). The static chamber consisted of a PVC base frame (20 cm width × 30 cm length × 15 cm height) with a water channel and a

removable cover (20 cm width \times 30 cm length \times 20 cm height). The cover box was equipped with a sampling outlet and a thermometer in the upper plane. The chambers were established in the between-row area of each microplot after plowing, and the base was inserted to a depth of 15 cm in the soil. When collecting gas samples, we filled the water channels with water to keep the chamber airtight.

Gas samples were obtained between 9:00 and 11:00 am. Five gas samples were taken at 0, 8, 16, 24, and 32 min after chamber covering for flux measurements, and an additional gas sample was obtained at 60 min closure time for $^{15}\text{N}_2\text{O}$ analyses. 35 and 15 ml gas samples were collected for flux measurements and $^{15}\text{N}_2\text{O}$ analyses, respectively, using 35-mL polypropylene syringes fitted with 3-way stopcocks. All the gas samples were stored in 12 ml evacuated vials (Labco, UK), and the vials for $^{15}\text{N}_2\text{O}$ analyses were helium-flushed. It was assumed that N_2O confined in the headspace at the time of chamber closure was equivalent to atmospheric N_2O and contained no excess ^{15}N .

The N_2O and CO_2 samples were analyzed within the sampling day using an Agilent 7820A gas chromatograph (Agilent Technologies Inc., SCLA, CA, USA), which was equipped with an electron capture detector (ECD) and a flame ionization detector (FID). The carrier gas for N_2O and CO_2 analysis was high-purity N_2 , and the buffer gas for ECD was 10% CO_2 in pure N_2 . The flow rates of the carrier gas were 25 and 30 mL min $^{-1}$ for the ECD and FID, respectively. Temperatures in the column ovens, ECD, and FID were set at 55°C, 330°C, and 250°C, respectively. The N_2O and CO_2 fluxes were calculated from the linear or nonlinear changes in gas concentrations

determined within the 32-min closure period (Hutchinson and Mosier, 1981; Yan *et al.*, 2013).

Fluxes of N₂O and CO₂ were measured daily for a week after fertilization events. The ¹⁵N₂O samples were also collected daily during the 7-day continuous sampling period after fertilization events (samples on the 5th day after top-dressing were missing because of rain), and additional ¹⁵N₂O samples were taken on the 10th day after top-dressing. For the non-fertilization period, only gas fluxes were measured, and the sampling was performed two times a week (samples were taken only once a week over winter).

The cumulative N₂O emissions were estimated by summing the daily mean fluxes of measurement and no-measurement days, with daily fluxes of no-measurement days being estimated as the arithmetic average of adjacent data (Huang *et al.*, 2013; Tian *et al.*, 2013).

2.4 ¹⁵N₂O analysis and calculation

The ¹⁵N abundances of N₂O samples were analyzed in the Stable Isotope Facility of the University of California at Davis. Stable isotope ratios of N were measured using a Thermo Scientific GasBench + Precon gas concentration system interfaced to a Thermo Scientific Delta V Plus isotope-ratio mass spectrometer (Thermo Electron Inc., Bremen, Germany).

The collected N₂O sample for ¹⁵N analysis contained a mixture of atmospheric and emitted N₂O. We used the following equation (Li *et al.*, 2016) to calculate the ¹⁵N

abundance (atom fraction ^{15}N) of emitted N_2O ($\text{atom}\% \text{ } ^{15}\text{N}_{em}$):

$$\text{atom}\% \text{ } ^{15}\text{N}_{em} = (\text{atom}\% \text{ } ^{15}\text{N}_{mix} \times C_{mix} - \text{atom}\% \text{ } ^{15}\text{N}_{air} \times C_{air}) / C_{em} \quad (1)$$

where $\text{atom}\% \text{ } ^{15}\text{N}_{mix}$ and $\text{atom}\% \text{ } ^{15}\text{N}_{air}$ are the ^{15}N abundances of headspace samples and ambient air (averaged 0.369% during the experiment), respectively; and C_{mix} , C_{air} , and C_{em} are the N_2O concentration of headspace samples, ambient air, and emitted N_2O respectively, and $C_{mix} = C_{air} + C_{em}$.

The proportion of N_2O flux derived from fertilizer ($\% \text{N}_2\text{O-N derived from applied N}$) was calculated according to the following equation (Nason and Myrold, 1991; Lampe *et al.*, 2006; Vallejo *et al.*, 2014):

$$\% \text{N}_2\text{O-N derived from applied N} = (^{15}\text{N}_{ap} \text{ } ^{15}\text{N}_{2\text{O}em} / ^{15}\text{N}_{ap} \text{ fertilizer}) \times 100 \quad (2)$$

where $^{15}\text{N}_{ap} \text{ } ^{15}\text{N}_{2\text{O}em}$ and $^{15}\text{N}_{ap} \text{ fertilizer}$ are the atom% excess of emitted N_2O ($\text{atom}\% \text{ } ^{15}\text{N}_{em}$ minus $\text{atom}\% \text{ } ^{15}\text{N}_{air}$) and ^{15}N labeled urea (10.21% minus $\text{atom}\% \text{ } ^{15}\text{N}_{air}$), respectively. The product of the total cumulative N_2O emissions and the $\% \text{N}_2\text{O-N derived from applied N}$ was calculated as cumulative fertilizer-derived N_2O emissions. The cumulative fertilizer-derived N_2O emissions after top-dressing may be from the top-dressing fertilizer and also the basal fertilizer, because we used ^{15}N labeled urea in both fertilization events.

2.5 Soil and plant sampling

In all microplots, soil samples were taken six times (i.e., before sowing, the 2nd day after basal fertilization, the 30th day after basal fertilization, the 5th day before top-dressing, the 2nd day after top-dressing, and on harvest). The dates of soil sampling

were shown in Fig. 1. On each soil sampling day, two soil cores (2.5 cm diameter) at 0–20 cm depth were taken within each microplot. Samples from the two soil cores were sieved (2 mm) and mixed well. The boreholes were refilled with PVC columns to avoid a change in gas exchange and water flow in the soil. The soil ammonium-N ($\text{NH}_4^+\text{-N}$) and nitrate-N ($\text{NO}_3^-\text{-N}$) were extracted from the fresh soils (20 g) in 100 mL of 1 M KCl solution and analyzed by a colorimetric continuous flow analyzer (AA3, SEAL Inc., Germany). At harvest, all the grain samples were thoroughly dried in a 65°C oven for the determination of crop yield (dry matter).

2.6 Statistical analysis

Differences in cumulative N_2O emissions, CO_2 emissions, and crop yield were determined by a *t*-test for least significant differences at $P < 0.05$. The values are expressed as arithmetic mean ($n = 3$) and standard error of the replications. The quadratic and linear model was used to estimate relationships between % N_2O -N derived from applied fertilizer N and the day after fertilization. SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

3. Results

3.1 N_2O and CO_2 fluxes

The peak N_2O emissions were mainly associated with N fertilization and/or irrigation events. The N1S0 and N1S1 treatments exhibited significantly higher N_2O fluxes

(peaking at 0.23–0.66 mg N₂O-N m⁻² h⁻¹) than the N0S0 and N0S1 treatments (peaking at 0.04–0.07 mg N₂O-N m⁻² h⁻¹; $P < 0.05$; Fig. 2). This significant ($P < 0.05$) increase of N₂O fluxes induced by fertilization lasted for about 7 days after basal fertilization and 10 days after top-dressing. Thereafter, N₂O fluxes of all treatments remained at < 0.02 mg N₂O-N m⁻² h⁻¹, and no statistically significant differences were found between N-fertilized and non-N-fertilized treatments ($P > 0.05$). In the basal fertilization period, the peak N₂O fluxes tended to be higher in N1S1 treatments (0.66 mg N₂O-N m⁻² h⁻¹; Fig. 2d) than that in N1S0 treatments (0.51 mg N₂O-N m⁻² h⁻¹; Fig. 2c). During the top-dressing period, the opposite trend was observed, i.e., 0.23 and 0.48 mg N₂O-N m⁻² h⁻¹ for the N1S1 and N1S0 treatments, respectively (Fig. 2d, c), although no overall significant difference in N₂O fluxes was found ($P > 0.05$).

During the basal fertilization/first irrigation event, straw incorporation strongly stimulated CO₂ fluxes both in non-N-fertilized plots and N-fertilized plots: peak values of CO₂ flux from N0S1 and N1S1 (115–127 mg CO₂-C m⁻² h⁻¹) were about 2.6-fold higher ($P < 0.05$) than those from N0S0 and N1S0 (39–52 mg CO₂-C m⁻² h⁻¹; $P < 0.05$; Fig. 3a, b). However, in the top-dressing/second irrigation period, this significant increase of CO₂ fluxes after straw-incorporation was only observed in N-fertilized plots (N1S1; Fig. 3b): the peak CO₂ fluxes in the N1S1 treatment (134.6 ± 7.92 mg CO₂-C m⁻² h⁻¹) was significantly higher than that in the N0S1 treatment (82.0 ± 7.84 mg CO₂-C m⁻² h⁻¹; $P < 0.05$).

3.2 Cumulative CO₂ and N₂O emissions and crop yield

In non-N-fertilized plots, the cumulative N₂O emissions of N0S1 treatments (368.20 g N₂O-N ha⁻¹) were 45% higher than those of N0S0 treatments (253.82 g N₂O-N ha⁻¹); however, in the N-fertilized plots, the cumulative N₂O emissions in the straw-incorporated and straw-removed treatments were similar (928.40 and 950.87 g N₂O-N ha⁻¹ for N1S1 and N1S0, respectively; Table 1). The N fertilization significantly increased the cumulative N₂O emissions by 152%–274% (928.40–950.87 vs. 253.82–368.20 g N₂O-N ha⁻¹; $P < 0.05$; Table 1).

Seasonal CO₂ emissions increased significantly after straw incorporation, and this was more apparent in non-N-fertilized treatments, i.e., N0S1 exhibited a 43% increase of CO₂ emission compared with N0S0 ($P < 0.05$; Table 1). No significant differences in seasonal CO₂ emissions were found between N-fertilized treatments (N1S0 and N1S1) and their corresponding non-N-fertilized treatments (N0S0 and N0S1; $P > 0.05$).

The crop yield of N1S0 and N1S1 treatments tended to be higher than that of non-N-fertilized treatments (N0S0 and N0S1, respectively), but the differences were not statistically significant ($P > 0.05$; Table 1). In N-fertilized plots, straw incorporation slightly increased the wheat yield, whereas in non-N-fertilized plots, the crop yields of the straw incorporation tended to decline, but no statistical differences were observed ($P > 0.05$; Table 1).

3.3 N₂O derived from the soil and fertilizer N

The proportion of N₂O fluxes derived from the fertilizer N reached maximum (55%–61%) on the 4th or 5th day after basal fertilization and then decreased to <50% on the 7th day after basal fertilization (Fig. 4a). This tendency was well described by a quadratic model ($P < 0.05$; $R^2 = 0.96$ and 0.92 for N1S0 and N1S1, respectively; Fig. 4a). According to this estimation, the percentage of daily N₂O emissions derived from fertilizer N was close to zero on the 10th day after basal fertilization. During the top-dressing period (7 to 17 Apr.), the percentage of fertilizer-derived N₂O reached maximum (56%–59%) on the 2nd day after fertilization and then declined afterwards (Fig. 4b). A linear model estimated that the proportion of fertilizer-derived N₂O was negligible on about the 20th day after top-dressing ($P < 0.05$; $R^2 = 0.60$ and 0.87 for N1S0 and N1S1, respectively; Fig. 4b). Straw incorporation had no significant effect on the ratio of fertilizer-derived N₂O fluxes ($P > 0.05$; Fig. 4). The cumulative fertilizer-derived N₂O emissions after basal fertilization were 209 and 210 g N₂O ha⁻¹ for N1S0 and N1S1 treatments (Fig. 5a), respectively, and in the top-dressing period, the corresponding N₂O emissions were 78 and 60 g N₂O-N ha⁻¹, respectively (Fig. 5b).

Fertilizer N-derived N₂O emissions accounted for 41.4%–53.8% of total emissions in the basal fertilization period and 51.8%–51.9% in the top-dressing period (Table 2).

The soil-derived N₂O emissions from N-fertilized plots (217–295 g N₂O-N ha⁻¹ after basal fertilization and 55–73 g N₂O-N ha⁻¹ after top-dressing) were significantly higher than those from non-N-fertilized plots (24–41 g N₂O-N ha⁻¹ after basal

fertilization and 30–31 g N₂O-N ha⁻¹ after top-dressing; $P < 0.05$; Fig. 5). This indicates that the N₂O emissions from the soil N pool were significantly promoted by the N fertilization. Straw incorporation tended to enhance N₂O emissions after the basal-fertilization (Fig. 5a) but decreased after the top-dressing period (Fig. 5b). However, straw incorporation had no significant effect on the cumulative N₂O emissions contributed by the fertilizer and soil N ($P > 0.05$; Fig. 5 and Table 2).

3.4 Soil N

Before the wheat was sown, soil NO₃⁻-N concentrations under the four treatments were all <10 mg kg⁻¹ (Fig. 6a). Application of N fertilizer significantly ($P < 0.05$) increased the NO₃⁻-N concentrations to 45.4–48.2 mg N kg⁻¹ during the basal fertilization period, and 25.8–32.7 mg N kg⁻¹ during the top-dressing period (Fig. 6a). On the 2nd day after top-dressing (9 Apr.), N1S1 was observed to have a remarkable effect of reducing soil NO₃⁻-N concentrations compared with N1S0; however, in other periods, no apparent differences of soil NO₃⁻ ($P > 0.05$) were detected between N1S1 and N1S0 treatments (Fig. 6a). Soil NH₄⁺-N concentrations always remained at a low level (<3.5 mg kg⁻¹), and there were no significant differences among treatments (Fig. 6b).

4. Discussion

4.1 Duration of N₂O emissions

The N₂O emission peaks occurred mainly after N fertilization events and lasted for approximately 1–2 weeks (Fig. 2), which is consistent with a number of recent studies (Bell *et al.*, 2015; Hinton *et al.*, 2015; Tan *et al.*, 2017; Yao *et al.*, 2017). This was mainly attributed to the high soil mineral N content after fertilization events (Ju *et al.*, 2011; Luo *et al.*, 2017; Zhang *et al.*, 2019; Fig. 6). In our study, the N₂O emission peaks occurring during the fertilization period (7 days after basal fertilization and 10 days after top-dressing), i.e., 578–620 g N₂O-N ha⁻¹, accounting for 59.6%–67.2% of the seasonal N₂O emissions (Fig. 2). Likewise, Ding *et al.* (2013) reported that up to 82%–98% of the fertilizer-induced N₂O emissions were emitted within the two weeks following fertilization. That is, although the growth period of winter wheat lasted for more than 240 days in the northern China, most N₂O was emitted in the initial 1–2 weeks following each fertilization event. This finding highlights that N₂O mitigation measures in the wheat season should mainly target the fertilization periods.

The proportion of fertilizer-derived N₂O fluxes declined to <50% since the 7th day after basal fertilization (Fig. 4a) and the 2nd–4th day after top-dressing (Fig. 4b).

Within 10–20 days after fertilization, fertilizer-derived N₂O became negligible (Fig. 4a, b). This could be explained by the reduced fertilizer-derived reactive N in soil due to microbial immobilization (Cai *et al.*, 2017), plant uptake (Omonode *et al.*, 2017), and losses through NH₃ volatilization (Xia *et al.*, 2017) and nitrate leaching (Huang *et*

al., 2017), etc. Similar findings were reported by a previous ^{15}N tracing study conducted in Europe (Linzmeier *et al.*, 2001). These results suggest that the duration of N_2O measurement to assess the fertilizer contribution is shorter than previously assumed. Intergovernmental Panel on Climate Change (IPCC) guidelines for estimating N_2O emission factors recommend that emission measurements are made for one year following fertilizer application (IPCC, 2006). Our research suggests that direct fertilizer emissions may occur over a period of weeks, and it may be appropriate to reassess the period over which emission factors are calculated for greenhouse gas inventory purposes.

4.2 Sources of N_2O emissions

Fertilizer-derived N_2O accounted for 41.4%–53.8% of the cumulative N_2O emissions in the fertilization period (Table 2), which was higher than regions in Europe (10%–40%; Linzmeier *et al.*, 2001) and Oceania (<4%; Di and Cameron, 2008). This was most likely to be related to the significantly higher N application rate in the intensively farmed region of northern China ($250 \text{ kg N ha}^{-1} \text{ season}^{-1}$ in our study) compared with Linzmeier *et al.* (2001) ($160 \text{ kg N ha}^{-1} \text{ season}^{-1}$) and Di and Cameron (2008) ($200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Our finding highlights that there is a great potential for lowering fertilizer-derived N_2O emissions by optimizing the N application rate in the study region.

Despite the high N application level in northern China, a large proportion (46.2%–58.6%) of soil-derived seasonal N_2O emissions was detected (Fig. 5), indicating a

high risk of N₂O generation from soil N. Background cumulative N₂O emissions (N₂O emissions from non-N-fertilized treatments) in our study were 0.25–0.37 kg N₂O-N ha⁻¹ season⁻¹ (Table 1), comparable to the values (0.22–0.47 kg N₂O-N ha⁻¹ season⁻¹) reported in previous site-specific studies (Cui *et al.*, 2012; Hu *et al.*, 2013; Huang *et al.*, 2013) and a meta-analysis conducted in the same region (Xu *et al.*, 2017). The results of this study showed that the soil-derived N₂O emissions in the fertilized plots were significantly higher than the background N₂O emissions ($P < 0.05$; Fig. 5), which could be attributed to the priming effect of N fertilizer on the soil N pool (Linzmeier *et al.*, 2001; Lampe *et al.*, 2006; Di and Cameron, 2008). This priming effect was most likely to have resulted from enhanced native soil N turnover induced by the increased microbial activity and root exudation (Kuzyakov *et al.*, 2000; Pearce, 2016; Liu *et al.*, 2017). Quantifying the contribution of fertilizer-derived N to the N₂O released by background emissions is a challenging task, but is important because background emissions are used in the calculation of emission factors (IPCC, 2006). However, these emission sources are difficult to separate in the field studies. Our findings indicate that the overall N₂O flux needs to be understood in the context of an interaction between fertilizer and soil N pools.

Farmland in northern China has received continuously high synthetic N applications (600 kg N ha⁻¹ yr⁻¹) over a long period (> 30 years). Consequently, large amounts of residual N have accumulated in soil (Cui *et al.*, 2013), which represents a large source of N₂O emissions in the subsequent crop season (Grant *et al.*, 2006). Our findings show that N₂O emissions induced by the priming effect accounted for 43.7%–87.6%

of soil-derived N₂O emissions (Fig. 5) and 21.0%–50.5% of the total emissions (Table 2), indicating that the risk of N₂O loss from the accumulated soil N could be intensified by N fertilization. In this case, optimum fertilization on the basis of soil residual N testing could be implemented in the region (Ju *et al.*, 2004; Wu *et al.*, 2014; Zhang *et al.*, 2014a) to increase N use efficiency and reduce the risk of N₂O loss from both fertilizer N and the soil N pools.

4.3 Impacts of straw incorporation on N₂O emissions

In N-fertilized treatments, straw incorporation tended to increase N₂O emissions in the basal fertilization period (Fig. 5a) but the opposite tendency was observed in the top-dressing period (Fig. 5b). For maize straw with a high C/N ratio (76:1 in our study), microbes would immobilize the N within soil to decompose the maize straw (Abalos *et al.*, 2013; Lin *et al.*, 2013; Lehtinen *et al.*, 2014). In the basal fertilization period, N uptake by plants was negligible, and soil N may be adequate for the decomposition of straw, as indicated by the similar CO₂ fluxes between N0S1 (Fig. 3a) and N1S1 (Fig. 3b) treatments (Esther *et al.*, 2014). Therefore, microbial N immobilization had no apparent effect on soil mineral N content after basal fertilization (Fig. 6). The increased N₂O emissions under straw-incorporated treatments (Fig. 5a) were probably derived from straw decomposition (Vigil *et al.*, 1991; Frimpong *et al.*, 2010). However, in the top-dressing period (at the jointing stage), significant increases of CO₂ fluxes after straw incorporation were only observed in N-fertilized plots (N1S1; Fig. 3b), suggesting that soil available N was the limiting factor of straw decomposition (López-

Bellido *et al.*, 2005; Song *et al.*, 2011; Chen *et al.*, 2014; Li *et al.*, 2017). The competition for available N between microorganisms and plants in straw-incorporated plots could have resulted in a decreased NO₃⁻-N concentration (Fig. 6a) and lower N₂O emissions (Fig. 5b). Our results demonstrate that crop straw interacts with N fertilization to control N₂O emissions in intensively farmed soils.

At the seasonal scale, when no N fertilizer was applied, 45% higher N₂O emissions were observed under the straw-incorporated (368 g N₂O-N ha⁻¹ season⁻¹) treatments relative to the treatments without straw (254 g N₂O-N ha⁻¹ season⁻¹); however, in N-fertilized plots, the N₂O emissions from straw-incorporated and straw-removed treatments were similar (928 vs. 951 g N₂O-N ha⁻¹ season⁻¹; Table 1). Similar observations were reported by previous meta-analyses (Shan and Yan, 2013; Xu *et al.*, 2017). In the non-N-fertilized soils where N₂O production was relatively constrained by the limited available N (Kim and Giltrap, 2017), straw input supplied about 60 kg N ha⁻¹ in our study (N% = 0.69), nearly the same level as soil mineral N quantity (77 kg N ha⁻¹, 0–100 cm; data not shown), which provided an important substrate for N₂O generation (Kumar and Goh, 1999; Chen *et al.*, 2013; Huang *et al.*, 2017). However, in the N-fertilized plots, N₂O emissions induced by straw N addition were probably overwhelmed by the intensive N fertilization (Yao *et al.*, 2017), although straw-incorporated treatments received about 24% higher total N input than straw-removed treatments. Our results suggest that straw incorporation could enhance the N supply without increasing the N₂O emissions in intensively managed soils.

It should be mentioned that the soil temperature during the wheat season in northern

China (10.8°C) is relatively low, which resulted in a moderate microbial activity and slow straw decomposition rate (Hartmann *et al.*, 2014; Warren Raffa *et al.*, 2015). Thus, it is probably not possible to critically examine significant effects of straw incorporation in just one cropping season. Further in-situ ¹⁵N tracer studies should be conducted to assess the long-term effect of straw incorporation on the rate and source of N₂O emissions.

Conclusions

This in-situ ¹⁵N tracing study provided an insight into the rate and source of N₂O emissions and the effect of straw incorporation on N₂O emissions in the intensively farmed soils of northern China. About 60%–67% of the wheat seasonal N₂O emissions were lost in the one to two weeks following fertilization events. Within 10–20 days after fertilization, fertilizer-derived N₂O became negligible, suggesting that it may be appropriate to reassess the period over which emission factors are calculated for greenhouse gas inventory purposes. Because of the long duration of high N input in this region, fertilizer N and soil N both accounted for about 40%–60% of the seasonal N₂O emissions in the fertilization period, which implies equivalent roles of the soil N pool and fertilizer N in N₂O generation in long-term intensively farmed soils. During the basal fertilization/first irrigation events, straw incorporation significantly stimulated CO₂ fluxes both in N-fertilized and non-N-fertilized plots; however, after the top-dressing/second irrigation events, the significant increase of CO₂ fluxes induced by straw incorporation was only observed in the N-fertilized

treatment. Application of N fertilizer had a significant priming effect on the soil N pool, which may increase the risk of N₂O loss from N accumulated in the soil. Straw incorporation interacted with N fertilization, and exhibited a tendency of enhancing N₂O emissions in the basal fertilization and lowering N₂O emissions in the top-dressing period. In N-fertilized plots, the seasonal N₂O emissions from straw-incorporated and straw-removed treatments were similar, indicating straw incorporation enhanced N supply without increasing the N₂O emissions. Our study highlights the necessity of examining the long-term impacts of N fertilization and straw incorporation on greenhouse gas emissions.

Acknowledgements

Special thanks to the anonymous reviewers for their helpful comments that significantly improved the manuscript.

Funding

This work was supported by the National Key Research and Development Program (grant number 2017YFD0800605 and 2016YFD0201200) and the Biotechnology and Biological Sciences Research Council (grant numbers BB/P01268X/1 and BB/N013484/1).

Declarations of interest: none.

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Figure captions

Figure 1 Dates of field management practices and sampling, and amounts of irrigation and N fertilizer applications during the experimental period.

Figure 2 Fluxes of N₂O under (a) N0S0, (b) N0S1, (c) N1S0, and (d) N1S1 treatments. Error bars represent standard error ($n = 3$). The solid arrows indicate ¹⁵N fertilizer application, and the dotted arrows indicate irrigation events.

Figure 3 Fluxes of CO₂ for (a) non-N-fertilized and (b) N-fertilized treatments. Error bars represent standard error ($n = 3$). Dotted and solid arrows indicate irrigation events and N fertilizer application, respectively.

Figure 4 Percentage of applied N-derived daily N₂O emissions after (a) basal fertilization (23 Oct.) and (b) top-dressing (7 Apr.) for the N1S0 and N1S1 treatments. ** represents 0.01 significance level. Error bars represent standard error ($n = 3$).

Figure 5 Cumulative N₂O emissions after (a) basal fertilization (23 Oct.) and (b) top-dressing (7 Apr.), which are divided into fertilizer-derived and soil-derived. Different capital and lowercase letters indicate significant differences of fertilizer-derived and soil-derived N₂O emissions, respectively, at $P < 0.05$. Error bars represent standard error ($n = 3$). Dashed lines and braces are used to indicate the additional N₂O release from the soil N pool after N fertilization.

Figure 6 (a) NO₃⁻-N and (b) NH₄⁺-N content from different sampling dates. Error bars represent standard error ($n = 3$). Arrows indicate irrigation events and/or N fertilizer application.

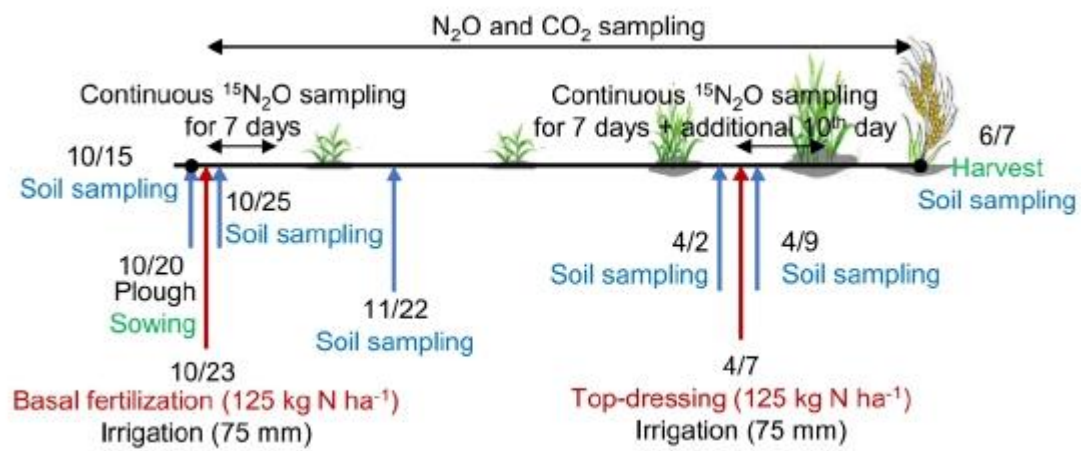
Table 1 Cumulative N₂O and CO₂ emissions and crop yield (dry matter). Data are expressed as mean \pm standard error ($n = 3$). Different letters indicate significant differences among the treatments at $P < 0.05$.

Treatment	N ₂ O emission (g N ₂ O-N ha ⁻¹)	CO ₂ emission (Mg CO ₂ -C ha ⁻¹)	Yield (g m ⁻²)
N0S0	253.82 \pm 51.57 b	1.46 \pm 0.08 c	700.15 \pm 54.11 a
N0S1	368.20 \pm 32.50 b	2.09 \pm 0.16 a	637.21 \pm 49.20 a
N1S0	950.87 \pm 150.67 a	1.64 \pm 0.12 bc	770.51 \pm 48.46 a
N1S1	928.40 \pm 79.89 a	1.96 \pm 0.03 ab	817.38 \pm 93.04 a

Table 2 Proportion of N₂O emissions derived from background, priming effect, and fertilizer. Data are expressed as mean \pm standard error ($n = 3$). Different letters indicate significant differences between different treatments at $P < 0.05$.

Event	Treatment	Background	Priming effect	Fertilizer
Basal fertilization	N1S0	5.74 \pm 0.36% a	40.47 \pm 8.33% a	53.8 \pm 8.18% a
	N1S1	8.17 \pm 1.54% a	50.47 \pm 6.53% a	41.38 \pm 800% a
Top-dressing	N1S0	19.60 \pm 0.89% a	28.61 \pm 4.59% a	51.81 \pm 3.74% a
	N1S1	27.07 \pm 2.97% a	21.02 \pm 1.54% a	51.93 \pm 2.35% a

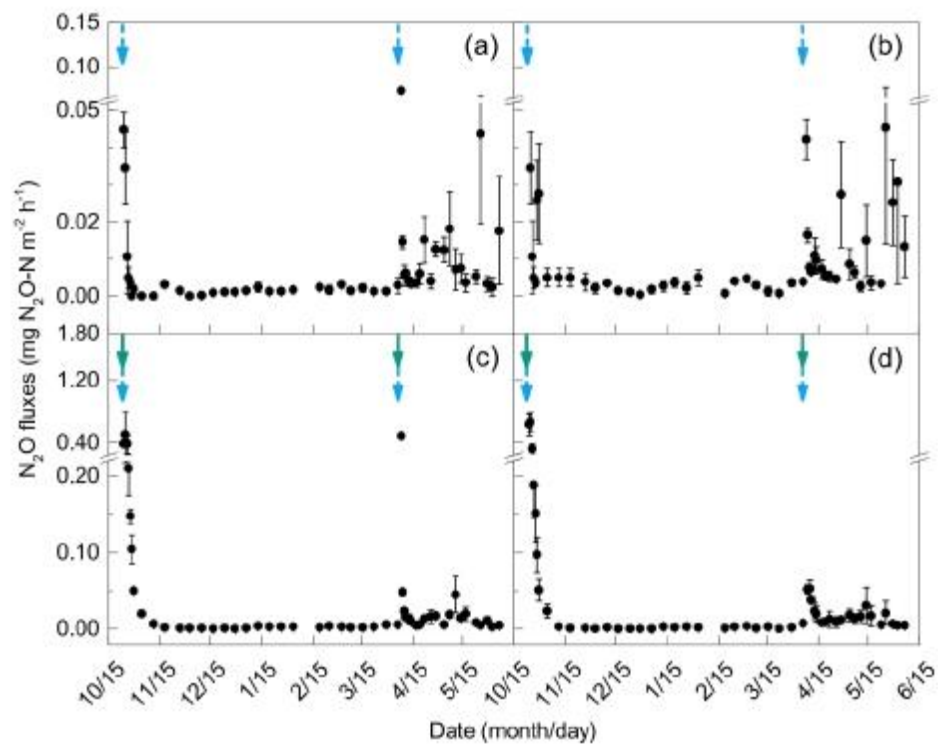
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861 **Fig. 2**



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Fig. 3

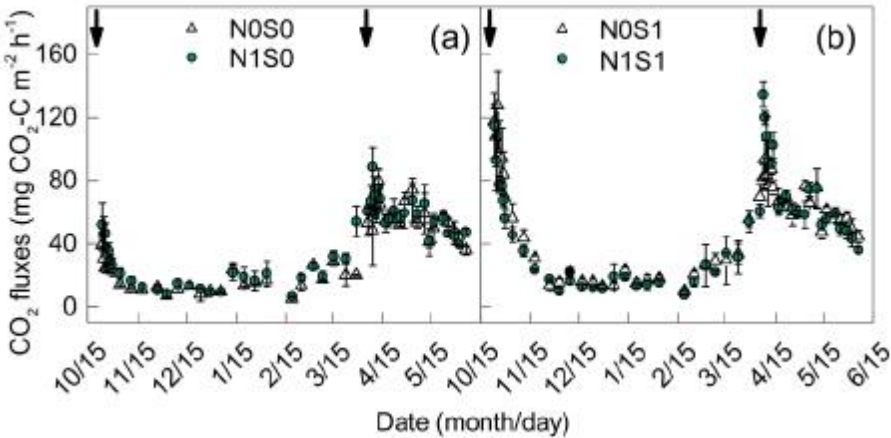


Fig. 4

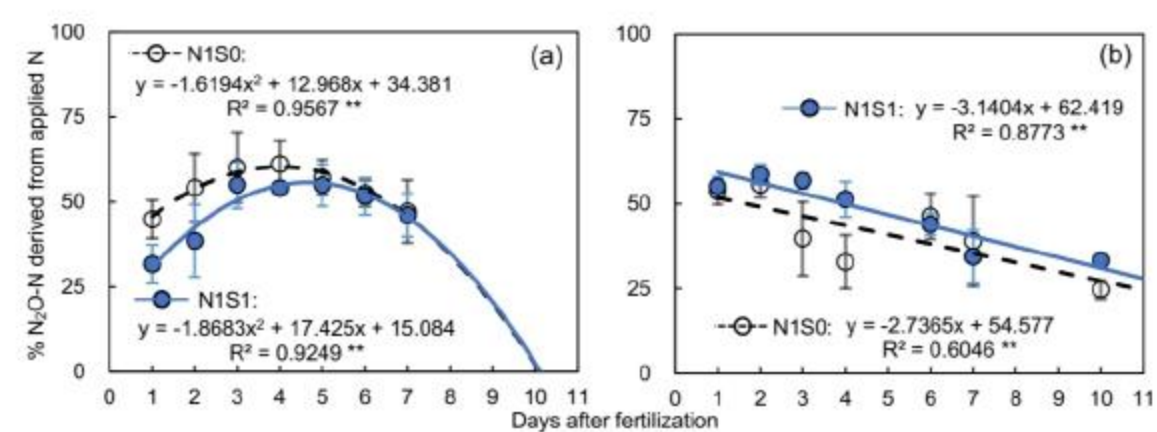
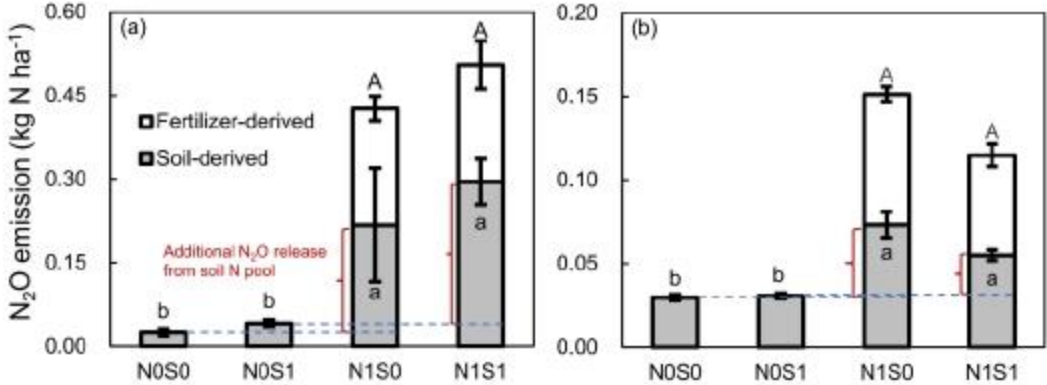


Fig. 5



873 **Fig. 6**

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